

# Neurobiology and behaviour: A network of connections

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*This article is dedicated to Dagmar von Helversen: mentor, friend and eternal inspiration, whose tragic and untimely passing away robbed us of one of the finest neuroethologists of our time.*

**Neurobiology is intimately related to animal behaviour at several levels. Mechanistically, the nervous system is the substrate that produces behaviour in response to external and internal stimuli. The structures and physiological properties of neurons and circuits not only determine immediate behavioural responses but also serve to both constrain and enable the evolution of different kinds of signals, behaviours and community structures. These issues have been explored in this article using acoustically communicating orthopteran insects as examples.**

**Keywords:** Acoustic communication, animal behaviour, crickets, insects, neurobiology.

THE dawn chorus of birds, owls swooping down on their prey in total darkness, military formations of ants moving their eggs and larvae to new locations, bees foraging for nectar in brightly coloured flowers, hawks hovering overhead looking for prey that freeze into immobility on detecting them: these are but a few of the innumerable examples of animal behaviour that we may encounter in our everyday lives. The moment we move from indifferent observation to asking *how* animals manage to do what they do and *why* they behave as they do, we enter the realm of the science of animal behaviour.

Every behaviour of every individual of any species is brought about by causal physiological processes that may be influenced by the current and immediate environment, the ontogenetic history of that individual, its evolutionary history and by the survival or adaptive value of that behaviour. The recognition of this fact is due to the great ethologist Niko Tinbergen, who carried out many pioneering studies in animal behaviour and proposed that any behaviour can only be completely understood when all four of the above aspects are taken into consideration<sup>1</sup>. Unfortunately, with but a few notable exceptions, the science of animal behaviour as we know it today has divided into two largely independent subdisciplines: neuroethology, which concerns itself with the physiological aspects

of behaviour, and behavioural ecology, which examines the evolutionary and ecological aspects of behaviour.

This article is concerned largely with neuroethology and explores the connections between neurobiology and behaviour: the nature of these connections, why understanding them is important to the study of animal behaviour as a whole, and the methods by which these connections may be explored. Most of the examples used to illustrate these points are taken from work on acoustically communicating orthopteran insects and vertebrates. This bias is purely for the convenience of presentation, and the general points derived are applicable across taxa, systems and behaviours.

## Neurobiology and behaviour: the obvious connection

Behaviour may be defined in a broad sense as the response of an individual or a group of individuals (whether or not it involves active movement) to external and internal stimuli. Such a definition includes, for example, phototropic responses of plants<sup>1</sup> and chemotactic responses of unicellular bacteria<sup>2</sup>, neither of which possess nervous systems. In the vast majority of multicellular animal taxa which are the subject of this review, however, it is the nervous system that underlies and mediates behaviour. The nervous system receives stimuli, both external and internal, transduces them into a common electrical code, processes the resulting information and then drives the somatic structures that provide the overt signature of behaviour. In other words, the nervous system is the direct causal agent of a process whose observable effect we term 'behaviour'. It follows that any in-depth understanding of behaviour will not be possible without understanding the structure and function of the underlying neuronal elements and circuits, and this is precisely the goal of neuroethology.

On the other hand, one may ask whether it is possible to study individual behaviour without examining the underlying neuronal circuitry. The answer is undoubtedly yes: ethograms, for example, provide quantitative descriptions of observed behaviours. Behavioural observations may also be used to develop correlative predictions, be they specific stimulus-response combinations or probabilistic

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predictions of future behaviour. Just as Mendel was able to infer the laws of heredity without understanding the structure or function of the chromosomes and genes that underlie hereditary transmission, it is possible to study behaviour in a purely correlative and statistical fashion and make reasonable inferences. But just as the study of chromosomes and genes has revealed the actual mechanisms of hereditary transmission, the reasons for the observed patterns, so also the study of the neural substrates of behaviour has greatly increased our understanding of what causes the observed behavioural patterns. As I will further argue, the neurobiology of behaviour, besides providing the immediate causal explanation for behaviour, is often valuable in terms of understanding its ecological and evolutionary aspects as well.

Acoustically communicating orthopteran insects provide excellent systems to explore the connections between neurobiology and behaviour. The advantage of these insect systems lies in their rich repertoire of relatively stereotyped behaviours that easily lend themselves to the development of behavioural paradigms, as well as their relatively large size that enables the performance of neurophysiological experiments. Among vertebrates, frogs provide a very similar system with many of the same advantages and it is no accident that these are among the best-studied taxa in neuroethology.

### Acoustic communication in orthopteran insects

The Order Orthoptera (crickets and grasshoppers) is one of the evolutionarily ancient orders of insects and is distributed worldwide, with several representative groups in both temperate and tropical regions of the Old and New World. The order may be broadly sub-divided into two suborders: the Ensifera (or crickets) and the Caelifera (or grasshoppers)<sup>3</sup>. Two of the major families of Ensifera include the Gryllidae (or true crickets) and the Tettigoniidae (or bushcrickets)<sup>3</sup>. Most ensiferan and some caeliferan taxa (notably the sub-family Gomphocerinae) use acoustic signals in the context of mate attraction. The calling songs are stereotyped and highly species-specific, with each cricket or grasshopper species producing a unique song<sup>4</sup>. The acoustic signals or calling songs are produced by adult males in order to attract females of the same species (conspecific females) from a distance<sup>4</sup>. In the case of most gryllid and many tettigoniid species, females lack stridulatory structures and do not produce sound. The species-specific songs of the males selectively attract conspecific females, who must detect and recognize the conspecific song pattern, localize the position of the sender and approach the male<sup>4,5</sup>. In the case of some tettigoniid and grasshopper species, duetting occurs between males and females, the onus of localizing prospective mates being placed on the male<sup>5,6</sup>. In these duetting species, females still require to detect and recognize conspecific

male calling songs, but their response consists of a reply song, which is in turn used by the male to localize the responding female<sup>5,6</sup>.

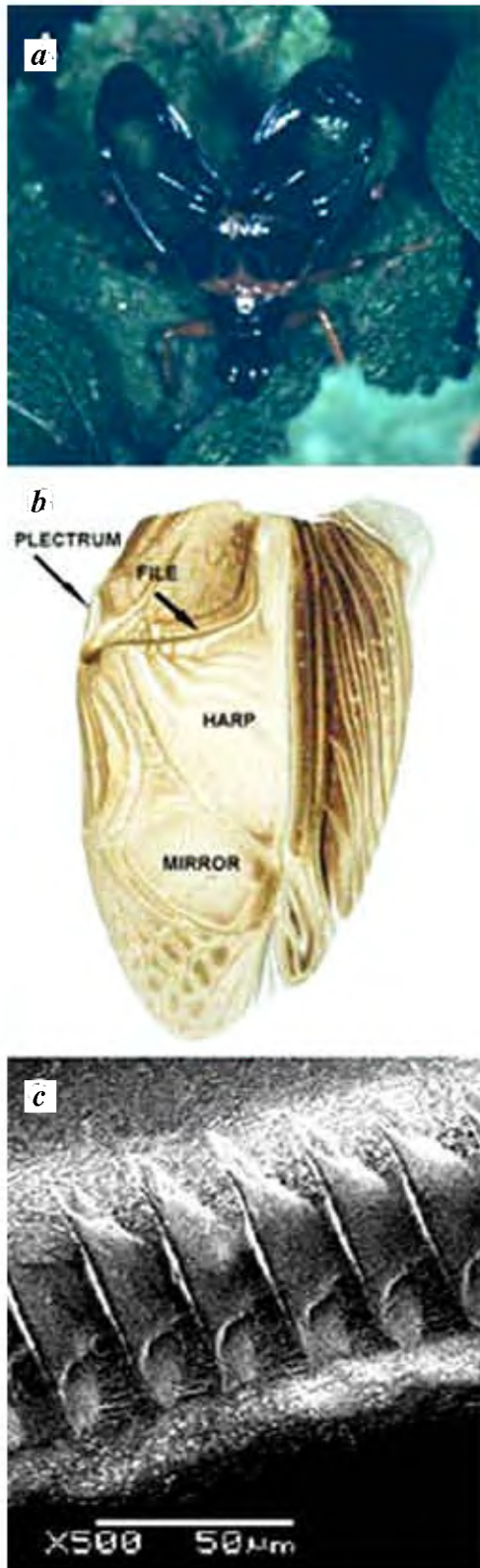
The neural basis of this selective phonotactic behaviour has been the subject of intensive study over decades and has involved two different methodological approaches. The neurophysiological approach involves recording the electrical activity of populations of neurons or single neurons in neuronal circuits that are hypothesized to underlie specific behaviours. These patterns of activity are then used to explain specific aspects of the behaviour being studied. On the other hand, the neural basis of the same behaviour may be studied using behavioural paradigms, in an elegant feedback manner. In the following paragraphs, I shall draw examples to illustrate the two approaches and bring out how they complement each other.

### Sound production and reception in orthopteran insects

Both gryllids and tettigoniids produce sound by rubbing the forewings together: a specialized structure called the plectrum (on the posterior margin of one forewing) scrapes against a row of pegs on the stridulatory file on the ventral surface of the other forewing to produce sound (Figure 1). The sound is then amplified by special resonating areas of the forewing, such as the harp and mirror. Caeliferans (grasshoppers) typically produce sound by rubbing the femur of the hind leg against a modified vein on the forewing.

In a large proportion of gryllid and tettigoniid species, both males and females possess tympanal ears, which are located, one on each foreleg, at the proximal end of the tibia. The axons of the auditory receptor neurons of ensiferans project from each ear through the femur of the foreleg to the prothoracic ganglion (TG1) of the central nervous system (Figure 2). Here they synapse both with local interneurons (whose axons are restricted to the prothoracic ganglion), and with ascending auditory interneurons that carry information to the brain<sup>7</sup>. Among local interneurons, the paired omega neurons (one on each side of the prothoracic ganglion) have been extensively characterized and probably play a role in sound localization<sup>4</sup>. Among ensiferans, there are only two ascending auditory interneurons on each side, often termed AN1 and AN2<sup>4</sup>. These project to the protocerebral region of the brain (Figure 2).

The auditory system of grasshoppers is organized somewhat differently than in crickets<sup>4,7</sup>. The tympanal ears of grasshoppers are located laterally on either side of the first abdominal segment and are hidden by the wings. Their auditory receptors correspondingly project to the metathoracic ganglion (TG3), where a considerable amount of neuronal processing occurs. In contrast to the ensiferans, grasshoppers possess at least fifteen pairs of

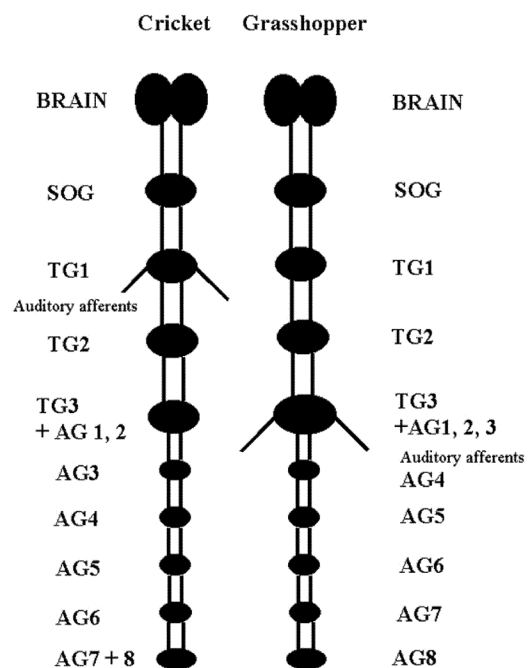


**Figure 1.** Mechanism of sound production in crickets. *a*, A singing field cricket (*Itaropsis* sp.), illustrating the raised forewings being rubbed together. *b*, Forewing of a field cricket (dorsal view) showing the stridulatory structures: The plectrum of one wing is rubbed against the file of the other to produce sound, which is amplified by the resonating structures, the harp and mirror. *c*, Scanning electron microscopic view of the ventral side of the file to show the stridulatory pegs.

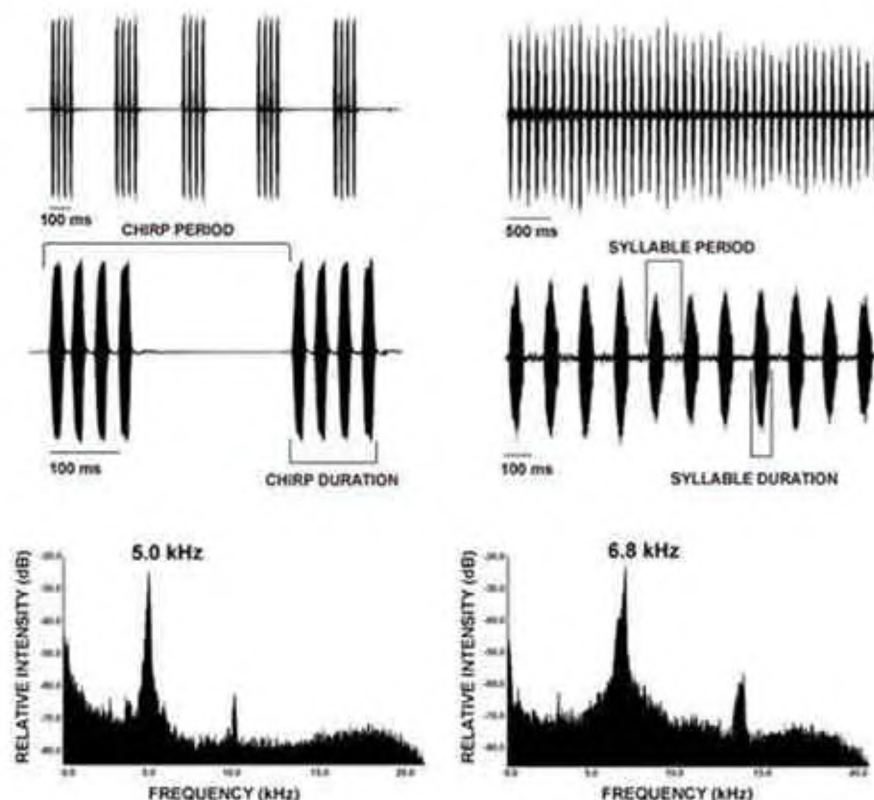
ascending auditory interneurons, instead of two<sup>4</sup>. The reason for this striking difference in numbers of ascending auditory interneurons between the Ensifera and Caelifera is not understood.

### Using neurophysiology to understand behaviour

Female crickets preferentially orient towards the calling songs of conspecific males even when they can simultaneously hear the calls of males of other species (heterospecific calls)<sup>8</sup>. The acoustic signals provide two major cues that could potentially be used by females to discriminate between song patterns. These include the frequency or spectral composition and the temporal pattern or rhythmic features. The spectra of the calling songs of gryllids are close to tonal, with most of the energy of the song concentrated in a narrow frequency band (the carrier frequency), typically between 2 and 8 kHz, depending on the species<sup>9</sup> (Figure 3). In true crickets, each closing stroke of the forewings produces a pulse or syllable of sound, whereas the opening stroke is silent, giving the song a discontinuous, pulsed character<sup>9</sup> (Figure 3). The repetition rates and durations of syllables are highly species-specific and provide cues to discriminate between different song patterns, if the nervous system could somehow measure them. In addition, the songs of some species possess more complex temporal patterns, wherein the syllables are grouped to form chirps, generating a second-order



**Figure 2.** Anatomy of the central nervous system of crickets and grasshoppers: A schematic representation showing the chain of ganglia and connectives that comprise the central nervous system. SOG, suboesophageal ganglion; TG, thoracic ganglion; AG, abdominal ganglion. Adapted from Elsner, 1994.



**Figure 3.** Structure of the calling songs of two field cricket species, *Gryllus bimaculatus* (left panels) and *Itaropsis* sp. (right panels). Top panel: Oscillograms (Y-axis represents sound amplitude and X-axis represents time) illustrating the difference in macro-temporal features between the two species: *G. bimaculatus* produces discontinuous chirps whereas *Itaropsis* produces a continuous trill. Middle panel: A magnified view to show the individual syllables constituting the chirps and trill. Bottom panel: Power spectra to illustrate the species-specific carrier frequencies of 5 kHz (*G. bimaculatus*) and 6.8 kHz (*Itaropsis* sp.) respectively.

structure<sup>9</sup> (Figure 3), with chirp rates and chirp durations, that could also be used for recognition and discrimination of song patterns.

#### *Frequency tuning of phonotaxis: Conspecific song and positive phonotaxis*

In the field cricket species *Teleogryllus oceanicus* and *Teleogryllus commodus*, which are morphologically indistinguishable sibling species, the carrier frequencies of the calling songs are 4.5 kHz and 3.5 kHz respectively<sup>10,11</sup>. Behavioural walking phonotaxis experiments in the laboratory have shown that *T. oceanicus* females do not approach calling songs played back at 3.5 kHz (the heterospecific carrier frequency), even if the conspecific temporal pattern is maintained<sup>12</sup>. Phonotaxis experiments in tethered flight also show that the tendency to approach a calling song model with the species-specific temporal pattern varies strongly with the carrier frequency<sup>13</sup> (Figure 4). Song models at the species-typical carrier frequency of 4.5–5 kHz are the most effective and the behavioural threshold of the phonotactic response (the minimum signal intensity or loudness required to elicit a significant

phonotactic response) is minimal at this frequency, rolling off fairly steeply on either side (Figure 4b). In other words, the female phonotactic response is tuned to the frequency of the conspecific calling song.

The basis of the frequency tuning of the response was revealed by neurophysiological experiments. Extracellular recordings from the tympanal nerve (which carries the auditory receptor axons) reveal the summed activity of the receptor neurons ('audiograms'): when the frequency tuning of the ear was measured in terms of the threshold of the electrophysiological response at different frequencies, it was found to reflect the tuning of the behavioural response<sup>14</sup>. The frequency tuning of the positive phonotactic behavioural response is thus explicable in terms of the frequency tuning of the population of auditory receptors in the ear.

#### *Frequency tuning of phonotaxis: Ultrasound and negative phonotaxis*

In the same tethered flight experiment described above, Moiseff *et al.*<sup>13</sup> found that ultrasonic sound signals (above 20 kHz), even those possessing the temporal pat-



tern of conspecific song, evoked robust negative phonotactic responses in *T. oceanicus* (Figure 4c). Females steered away from such sounds, presumably because they are indicative of the presence of bats, which are predators of crickets. Thus, the same song pattern that elicited positive phonotaxis when presented at a frequency of 5 kHz was avoided when presented at 30 kHz. These behavioural experiments suggested a parallel processing of frequency information in the auditory system, with a low-frequency pathway mediating positive phonotaxis and a high-frequency pathway mediating negative phonotaxis. Again, electro-

physiological recordings have shown the presence of individual receptor cells tuned to different frequencies, with a large proportion (75%) tuned to the conspecific song frequency and a small proportion tuned in the range 10–12 kHz (8%) and ultrasonic frequencies (17%)<sup>15</sup>. At the level of the ascending interneurons, single-cell recordings revealed that the AN1 was tuned to the calling song frequency, whereas the AN2 was mainly sensitive to high frequencies, suggesting that this neuron was part of the pathway that mediated negative phonotaxis<sup>16</sup>.

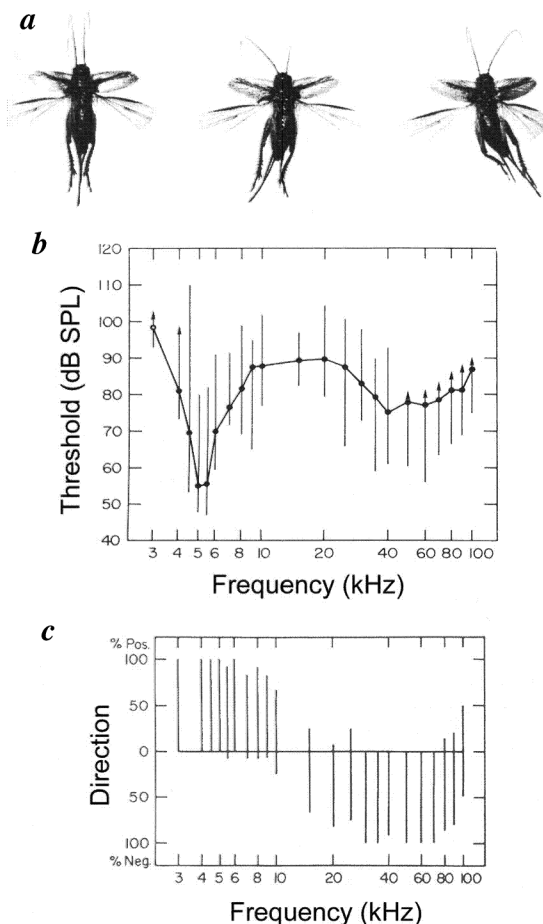
This was demonstrated by Nolen and Hoy<sup>17</sup> in an elegant (and difficult!) experiment wherein they recorded intracellularly the activity of the AN2 neuron in a tethered flying cricket and monitored the behavioural steering responses by measuring the electrical activity of the relevant muscles. By hyperpolarizing the AN2 neuron (preventing action potential generation) they were able to suppress the behavioural response to ultrasonic stimuli; on the other hand, eliciting a high rate of firing of action potentials in the AN2 by electrical stimulation produced the appropriate phonotactic response in the absence of acoustic stimulation. Thus the AN2 was shown to be both necessary and sufficient to drive the negative phonotactic response. Although the role of the AN1 has not been tested in the same manner due to the technical difficulty of recording from this interneuron, its frequency tuning characteristics suggest a major role in positive phonotaxis, lending support to the idea of two pathways mediating positive and negative phonotaxis respectively.

Negative phonotaxis is a very robust behaviour of obvious survival value (avoidance of bat predators) and the very small number of auditory receptors tuned to ultrasound therefore presents an interesting paradox. Fossil evidence suggests that sound production in crickets evolved at least as early as the Permian period and sounds were probably used to attract mates then as now<sup>14</sup>. Bats evolved in relatively recent geological times (the Eocene epoch) and their arrival probably imposed a strong selection pressure on crickets to evolve ultrasonic hearing and avoidance responses. This was possibly achieved by modification of the properties of a pre-existing auditory system that was adapted primarily for positive phonotaxis to low-frequency conspecific song<sup>14</sup>.

Pollack<sup>18</sup> showed that, although the number of ultrasound receptors may be small, the effect of activity in each individual receptor neuron on postsynaptic interneurons is disproportionately large (as compared with receptors tuned to lower frequencies), allowing a strong and robust behavioural response.

#### Tuning to the temporal pattern of conspecific song

As stated earlier, the temporal features of song could also potentially be used by females to recognize specific song patterns. Studies on a number of cricket species have indi-



**Figure 4.** The frequency tuning of positive and negative phonotaxis. **a**, Phonotactic steering responses of a female cricket (*Teleogryllus oceanicus*, dorsal view) in tethered flight in response to playback of song stimuli from a loudspeaker on her left side. Left, no acoustic stimulus; middle, conspecific calling song at 5 kHz; right, conspecific calling song at 30 kHz. The direction of inclination of the abdomen and ovipositor is indicative of the direction in which the cricket is attempting to turn. **b**, Threshold of the phonotactic response at different frequencies (see text for details). Note the sharp tuning to 4.5 kHz (the carrier frequency of the conspecific calling song) and the high threshold at 3.5 kHz (carrier frequency of *T. commodus* song). Arrowheads indicate that some animals had thresholds higher than the maximum stimulus intensity presented. **c**, The reversal of response from positive to negative phonotaxis as a function of stimulus frequency. Reprinted with permission from Moiseff, Pollack and Hoy, 1978, *PNAS, USA*, vol. 78, pp. 4052–56 (Ref. no. 13; copyright 1978 Moiseff, Pollack and Hoy).

cated that this is indeed the case<sup>5</sup>. The most thoroughly investigated feature is the syllable repetition rate. In species of the genus *Gryllus*, behavioural phonotaxis experiments in the laboratory, using playback of a number of song models in which the syllable repetition rate was systematically varied (while keeping the song energy constant), showed that there was a broad tuning of the female positive phonotactic response, with species-typical values of repetition rate eliciting the maximum response<sup>19</sup>.

Electrophysiological recordings revealed that, at the level of the ascending neurons AN1 and AN2, there was no evidence for a filtering of syllable rates: all recorded interneurons represented the syllable patterns in terms of bursts of action potentials corresponding to every syllable, but their responses were similar to stimuli of any repetition rate<sup>19</sup>. This suggested that syllable rate filtering occurred at a later stage of processing in the brain. In a series of classic experiments, Schildberger<sup>20</sup> used a combination of intracellular recordings of single protocerebral brain neurons and subsequent study of the anatomy of the same neurons to identify the candidate repetition rate filter elements. He found three interesting types of neurons: low-pass, high-pass and band-pass. The low-pass neurons responded selectively to stimuli with repetition rates lower than the optimum rate, the high-pass neurons responded to stimuli with rates higher than the optimum, while the band-pass neurons showed a tuning to the range of repetition rates that corresponded with the tuning of the behavioural response of the female. He thus proposed a model wherein the outputs of low-pass and high-pass filter neurons are ANDed to produce the tuned characteristics of band-pass neurons (that is, a certain minimum level of activity was required in both the low-pass and high-pass input neurons in order to activate a postsynaptic band-pass neuron on which they presumably converged).

The tuning of female responses to species-specific repetition rates may thus be explained by neurons and circuits in the brain whose physiological and anatomical properties allow them to function as band-pass filters for syllable repetition rates.

### Using behaviour to understand neural processing

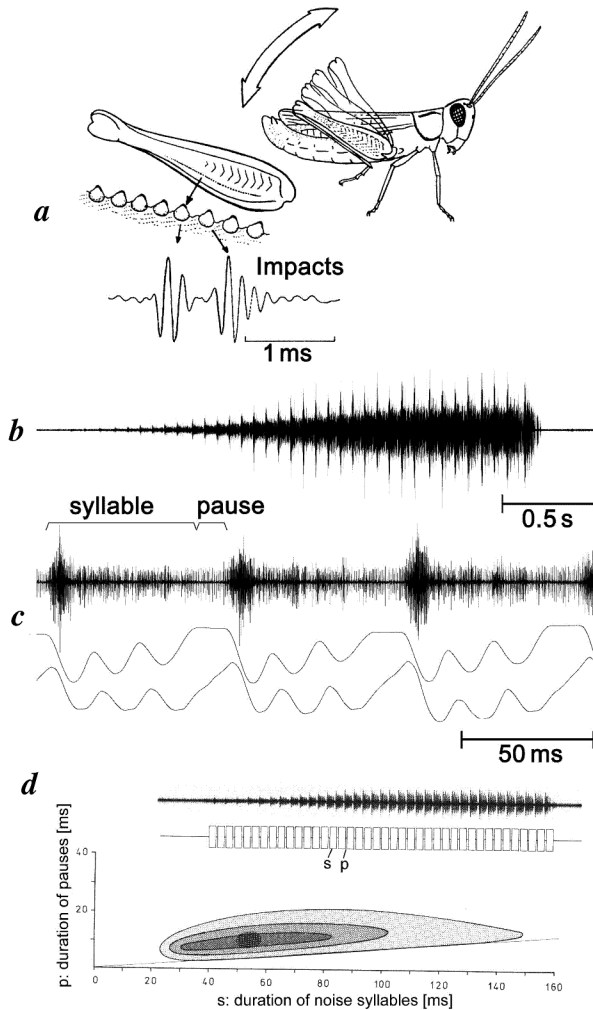
The usefulness of neurobiology in understanding behaviour is almost self-evident: much less obvious is the fact that the study of behaviour and the use of behavioural paradigms can contribute significantly to our understanding of neural processing. One of the most important and outstanding contributions to neurobiology was, in my opinion, made by an ethologist who is little known and rarely acknowledged. Alfred Seitz was an ethologist who made a number of careful observational studies on the aggression behaviour of the cichlid fish, *Haplochromis*<sup>21,22</sup>. Using a series of dummies or models of conspecific males, Seitz discovered the concept of key or sign stimuli, that is, only

certain key features of the models were used by the nervous system of the responding fish in order to recognize a rival and release a response. After a number of careful experiments wherein he compared the releasing effects of different combinations of sign stimuli, as well as their individual effects, Seitz came up with three important conclusions. First, each sign stimulus had a certain releasing value, which remained the same no matter which other stimuli it was combined with. Second, the releasing effect of the model depended on the sum of releasing effects of the individual stimuli. Third, a given level of behavioural response could result from different combinations of sign stimuli. From these observations, he formulated the Law of Heterogeneous Summation or the 'Reizsummenregel', which states that the effects of qualitatively different sign stimuli add together in the nervous system in a purely quantitative fashion and can replace each other quantitatively<sup>23</sup>. Phrased in modern terms, Seitz had discovered the concepts of neuronal integration and synaptic weighting so central to much of current neurobiology. Integration in neurons is mediated by graded local potentials (excitatory and inhibitory postsynaptic potentials). The nature of these graded potentials and their role in neuronal integration was discovered only decades later by neurophysiologists.

### *Song pattern recognition in the grasshopper Chorthippus biguttulus*

*Chorthippus biguttulus* is an acoustically communicating grasshopper in which pair formation is achieved by duetting between the sexes. Males produce phrases of calling songs, which are recognized by females, who respond with a response song. The response song of the female (which is different in structure from the male song) is in turn recognized by the male and used to localize the female<sup>6</sup>. The male song consists of phrases 2–3 s in length. The phrase of the male song is constituted by a series of repeated syllables separated by pauses (Figure 5). Syllable durations range from 40 to 100 ms and pause durations from 8 to 25 ms, depending on the ambient temperature<sup>24</sup>. Stridulation occurs by moving the two hind legs up and down such that a row of pegs on the inner side of each hind femur rubs against a specialized vein on the forewing to produce sound. Each peg strike results in the production of a very short click or impact (250–450  $\mu$ s) and each syllable is composed of a series of clicks (Figure 5). A syllable is produced by three successive down-up strokes, with the two hind legs moving slightly out of phase: this results in the camouflage of the brief silent gaps at the reversal points of the up-down movement. Each syllable is followed by a short pause: during this time, one of the hind legs is motionless, but the second continues to move, so that the resultant pauses are not silent, but noisy<sup>25</sup> (Figure 5).

Over the past few decades, the duetting response of the female has been used as a useful tool in behavioural playback experiments aimed at understanding the neural mechanisms of song pattern recognition. Using this approach, Helversen and Helversen<sup>6,26,27</sup> identified several of the song features that were important for recognition. These include a minimum syllable duration of about



**Figure 5.** Calling song of the grasshopper *Chorthippus biguttulus* and female responses to calling song models. *a*, Sound production occurs by rubbing a row of pegs on the inner side of the hind femur against a modified wing vein. Each peg strike produces a short click or impact of sound (250–450  $\mu$ s in duration). *b*, Oscillogram (plot of sound amplitude versus time) of a phrase of calling song. *c*, A magnified view of a section of the phrase to illustrate the syllables and noisy pauses. The line traces beneath show simultaneous recordings of the leg movements using an opto-electronic device: upward deflections indicate upstrokes, downward deflections are downstrokes. Note that the two legs are out of phase and that the lagging leg continues to move during the pause. *d*, The graph illustrates the optimal combinations of syllable and pause durations required to elicit female responses in playback experiments. The contour lines correspond to 20%, 50% and 80% responses respectively. *a*, *b*, *c* reprinted with permission from Balakrishnan, von Helversen and von Helversen 2001: Figure 1, p. 256 (Ref. 29; copyright 2001 Springer Verlag). *d* reprinted with permission from von Helversen and von Helversen 1983: Figure 2, p. 98 (Ref. no. 6; copyright 1983 Springer Verlag).

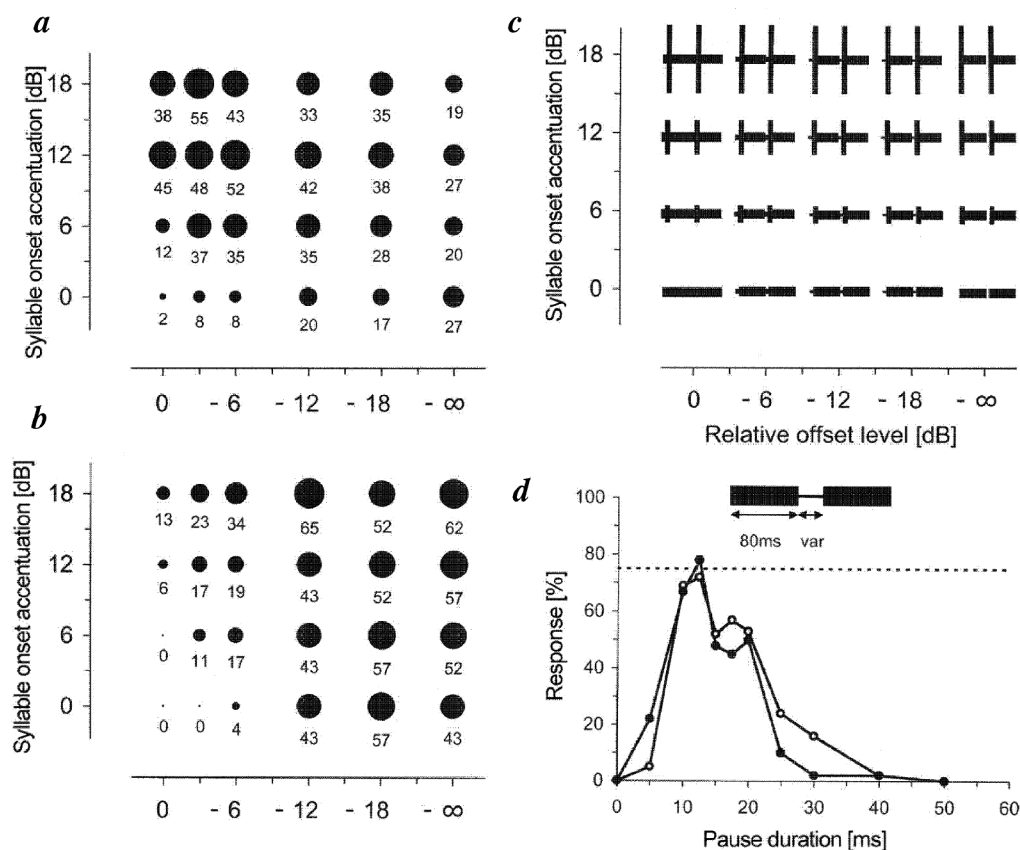
40 ms, an optimum syllable-pause duration ratio of about 5 : 1, a minimum phrase length of about one second, the absence of silent gaps within syllables, and an optimum syllable shape consisting of enhanced syllable onsets. In contrast to many cricket species, there was no tuning of the response to either the syllable duration (Figure 5) or the syllable repetition rate<sup>6,26</sup>, which were acceptable over very large ranges of values. On the other hand, female responses were tuned to the duration of the syllable pauses, indicating that pause durations were important for song recognition (Figure 5).

The measurement of pause durations implies the measurement of syllable onset and offset times. An exhaustive search that involved examining the neurophysiological properties of sixteen ascending auditory interneurons, as well as several local metathoracic interneurons, resulted in the discovery of a neuron, AN12, an ascending interneuron, whose properties suggested the function of syllable onset detection<sup>28</sup>. AN12 fired briefly at the onset of every syllable and could therefore be used to mark syllable onsets by any downstream circuitry dedicated to pause detection. In contrast, no neuron at any level examined exhibited physiological properties that reflected the coding of syllable offset time<sup>28</sup>.

Balakrishnan *et al.*<sup>29</sup> used a behavioural approach to the problem of pause detection: they found that syllable onset and offset detection was dependent on the relative sound intensity levels between syllables and pauses. Syllable onset and offset detection appeared to be parallel, independent processes. A careful evaluation also revealed enormous inter-individual variation in the optimal combinations of relative syllable onset and offset levels preferred by individual females of the species. Some females preferred high relative offset levels, others high onset levels, and yet others responded best in the absence of syllable offset cues (Figure 6): they preferred stimuli with no pauses as long as onsets were clearly marked<sup>29</sup>. Interestingly, these same females showed the typical tuning to a restricted range of syllable pause durations when stimuli with silent syllable pauses were presented to them (Figure 6 *d*). In other words, individual females that responded in the complete absence of pauses still had the ability to detect syllable offsets and reject long, silent pauses. This interesting and somewhat paradoxical observation, together with the large inter-individual variation, led us to develop a neuronal model of syllable-pause detection that did not involve syllable offset detection<sup>29</sup>. This neuronal model of pause detection, based on behavioural studies, is open to neurophysiological verification.

#### Parallel processing of song recognition and orientation

The auditory system of a male grasshopper has to recognize as well as localize the female's reply song in order to



**Figure 6.** Individual variation in the optimal values of temporal pattern features in *Chorthippus biguttulus*. **a**, Response profile of a female that preferred high syllable onset accentuations and no pauses. **b**, Response profile of a female that preferred high offset levels. Circles represent the per cent response (values shown as numbers below each circle) of an individual female to song patterns differing in syllable onset and offset levels (illustrated in **c**). Syllable onset accentuation: relative sound level of the first 10 ms of syllable with respect to the rest of the syllable. Relative offset level: sound level of the pause with respect to the end of the syllable. **d**, Response of an individual female to model song patterns (using rectangular syllables) differing in the duration of syllable pauses, illustrating the tuning of the response. Open circles, 64 dB and closed circles, 76 dB SPL (decibels sound pressure level). The dotted line shows the response of the same female to a stimulus containing enhanced syllable onsets and no pauses (see text for details). Reprinted with permission from Ref. 29: Balakrishnan, von Helversen and von Helversen 2001, Figs 7, 8 (p. 261, 262); copyright 2001 Springer Verlag).

locate the female. On hearing and recognizing a female reply song in response to his own, the male turns abruptly to the side from which the female's song comes and moves forward a short distance. He then sings again and turns after the next reply: since grasshoppers only lateralize (discriminate left from right) rather than actually localize sounds, the male's approach is in the form of a series of turns and jumps<sup>30</sup>. Further, the turning response and phonotactic approach of a male grasshopper are only elicited by female songs: males discriminate female songs on the basis of their ramped, triangular syllable shapes as compared to male syllables, which are rectangular<sup>26</sup>. A phrase of female reply song typically consists of six syllables.

The relation between the processes of song recognition and orientation was first investigated in females with the elegant split-song paradigm developed by Dagmar von Helversen<sup>31</sup>. The paradigm was, however, repeated several years later with males<sup>32</sup> because they show robust turning

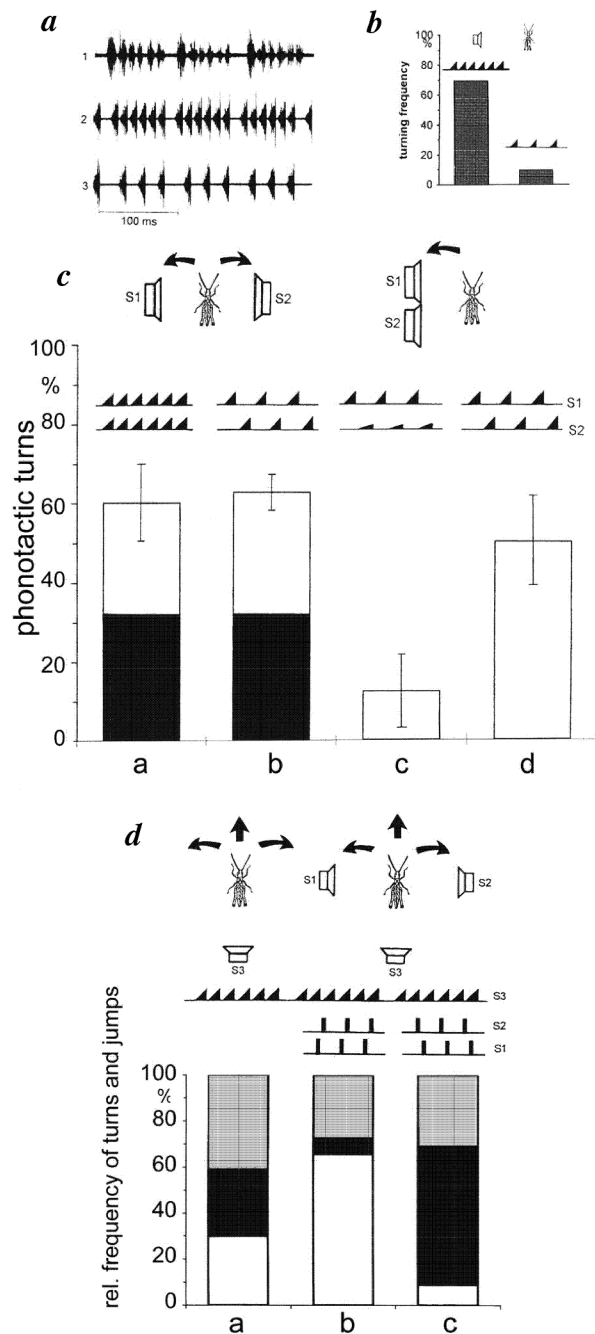
responses to playback of conspecific female reply songs<sup>32</sup>. In this experiment, male *Chorthippus biguttulus* were simultaneously presented with two ineffective versions of the female reply song, each consisting of a reply song with every second syllable omitted (Figure 7). The two ineffective song patterns were complementary, such that, if played together, the syllables of the two patterns were inter-leaved to produce the effective pattern of six syllables. When the two patterns, each ineffective on its own, were played from the same side, the grasshopper, as expected, turned towards that side (Figure 7c). The crucial situation was when the two ineffective patterns (played at intensities just above threshold) were played from either side of the animal. If the grasshopper had two independent neural representations of the song pattern, one on each side, it follows (since acoustic leakage to the other side was minimal), that the two ineffective patterns played from either side should elicit no response. In fact, grass-

hoppers did respond at normal levels to these split songs (Figure 7c), indicating that the pattern recognition circuitry pools the information from both sides. This would result in the loss of directional information, and lateralization must therefore involve a parallel pathway.

Further behavioural experiments were used to demonstrate this: when males were stimulated by female reply songs played from behind, the result was an equal proportion of jumps to the left, right and forward (Figure 7d). This was expected, since the stimulus was attractive but provided no directional cues. When a pair of clicks, one from each side and with a consistent delay between the two sides, was presented along with the female reply song, the proportion of jumps was biased towards the side of the leading click (Figure 7d: the precedence effect, see below). This indicates that the click, a completely ineffective stimulus on its own, was able to influence the direction of the jumps, again supporting the idea of a parallel pathway for orientation. This notion is supported by electrophysiological data, which show that interneurons that best encode song pattern features are direction-insensitive, whereas neurons whose responses are strongly influenced by direction show poor encoding of stimulus features<sup>30</sup>.

#### Localization of the neural circuits for song recognition and production

Since grasshoppers are poikilotherms (that is, they do not regulate their body temperature), the values of several song features such as syllable and chirp repetition rates and durations change with temperature<sup>24</sup>. Since these features are used for species recognition, this constitutes a problem, which has been resolved, in most acoustically communicating poikilotherms, by a corresponding change in the preferred rates and durations of the temporal pattern recognizers of the receivers<sup>33</sup>. For example, the preferred repetition rate for a female at 21°C would correspond to the rate produced by a male at that temperature. This phenomenon was exploited in an interesting behavioural experiment by Bauer and Helversen<sup>34</sup>, aimed at localizing the circuits for recognition and production. The head of a *Chorthippus parallelus* female was selectively heated five to nine degrees above the temperature of the thorax using a heating wire (with thermocouples monitoring head and thorax temperatures). The female was then presented conspecific male songs corresponding to different temperatures. Females responded best to male songs whose pattern corresponded to the temperature of the female's head and not the thorax, suggesting that the pattern recognition circuitry was localized to the brain. Conversely, the song pattern produced by the replying female corresponded to that of the temperature of her thorax and not the head, indicating that the song production circuits were localized to the thorax<sup>34</sup>. Both these observations have been confirmed by neurophysiological studies<sup>30</sup>.



**Figure 7.** The parallel processing of song recognition and orientation in the grasshopper *Chorthippus biguttulus*. **a**, Oscillograms of female song (1) and the two model songs (2 & 3) used for playback. The graphs (**b**, **c**, **d**) show the turning probability of males in response to presented stimuli. **b**, A model female reply song is effective, whereas a reply song lacking alternate syllables is ineffective in eliciting male turning responses. **c**, Two ineffective song models presented simultaneously (via speakers S1 and S2) are effective, irrespective of the relative position of the speakers (**b**, **d**: see text for details); white and black bars represent the proportion of turns to left and right sides. **d**, (**a**). The proportion of turns to the left (white) and right (black) sides and forward jumps (grey) in response to female reply song played from behind via speaker S3. When pairs of clicks are additionally presented, one from each side, the proportion of turns is biased to the side of the leading click (**b**, **c**). Adapted and reprinted with permission from Ref. 32: von Helversen and von Helversen 1995; Figs 2, 3 (p. 770, 771); copyright 1995 Springer Verlag.



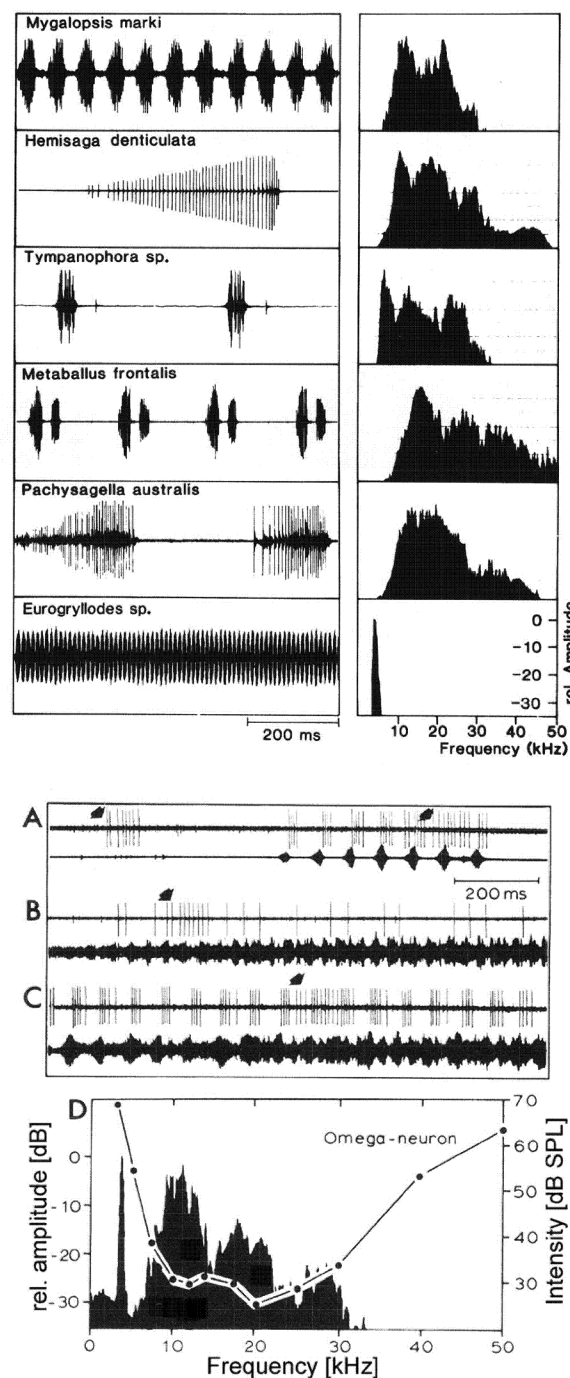
### Neurobiology and the ecology of acoustic communication

All of the experiments described so far have dealt with the problem of recognizing acoustic signals under ideal conditions. In actual fact, most animals have to communicate under extremely sub-optimal conditions imposed by the natural environment. Two of the major problems faced by acoustically communicating animals include signal degradation and interference. Signal degradation is caused by the habitat, including reflection and scattering from the ground and vegetation, which degrades both spectral and temporal characteristics of the signal<sup>35</sup>. Interference and masking of signals is largely due to other biotic sources of noise, the most common being the calls of other acoustically communicating species<sup>35</sup>.

The observations made using behavioural and neurophysiological experiments in the laboratory under ideal conditions give us no information on, and do not necessarily reflect, the responses under natural conditions. To better understand how information is represented and processed by the nervous system under realistic, natural conditions, a few neuroethologists have taken their neurophysiological set-ups out into the field. The pioneering work in this area of sensory ecology was carried out by Kenneth Roeder<sup>36</sup>, who made recordings from the ears of restrained moths placed outside in the field in order to find out at what distance and intensity a moth first got to hear the ultrasonic pulses emitted by its bat predators. This approach of using the ear of the animal as a 'biological microphone' placed outside under natural conditions, together with electrophysiological recordings, has been invaluable in providing insights into the actual environment as perceived by the animal. With respect to cricket acoustic communication, the use of this technique was pioneered by Heiner Römer in a series of elegant studies, some of which will be described below<sup>35,37</sup>.

#### Interspecific interference: temporal and frequency partitioning

The problem of interspecific interference arises when several species call together in the same habitat. Römer *et al.*<sup>37</sup> studied this phenomenon in a woodland community of six ensiferan species near Perth, Western Australia. Five of the six species were tettigoniids whose broadband calling songs (about 6–30 kHz) showed considerable overlap in their spectral features. The sixth species was a gryllid whose calling song showed a typical narrow-band spectrum at 3.5 kHz (Figure 8). By recording the pattern of activity of action potentials from the omega neuron (a local prothoracic auditory interneuron whose frequency tuning reflects that of the ear) of the tettigoniid *Mygalopsis marki*, they showed that the representation of the conspecific chirp pattern of a *M. marki* male (calling



**Figure 8.** Interspecific interference in acoustic communication: a neurophysiological view. The top panel shows oscillograms and power spectra of six cricket species co-existing in a woodland community. The middle panel shows three pairs of traces (A, B and C): the top trace in each shows the spiking activity of an auditory interneuron (the omega neuron) of an *M. marki* male recorded in the field. The lower trace in each is the sound recording of ambient calls. The arrows indicate when the neuronal representation of the conspecific *M. marki* call is obscured by a simultaneously calling *H. denticulata*. The bottom panel (D) shows the threshold tuning curve of the activity of the omega neuron in response to different frequencies; this is superimposed on the power spectrum of the simultaneously calling species. Note that the song of the gryllid *Eurogryllodes* at 3.5 kHz is filtered out by the omega neuron. Adapted and reprinted with permission from Ref. 37: Römer, Bailey and Dadour 1989; Figs 2, 4 (p. 612, 613); copyright 1989 Springer Verlag.

6.5 m from the preparation) was destroyed whenever there was a chirp from a neighbouring calling male of the species *Hemisaga denticulata* (Figure 8 a–c). Thus, the call of *Hemisaga denticulata* degraded the neuronal representation of the conspecific call in *M. marki* and the converse is also likely to be true.

In fact, the two species appeared to partition their calling times within a night in order to avoid acoustic interference: this was suggested by two lines of evidence. In one of the sites, observations of calling activity indicated that *H. denticulata* was active mostly between 4 and 8 p.m. whereas *M. marki* called mostly after 8 p.m. In a second site, in the absence of *M. marki*, *H. denticulata* extended its calling time until 10 p.m. That these temporal calling patterns were driven by the need to minimize acoustic interference was also demonstrated in the field in a manipulative experiment<sup>37</sup>.

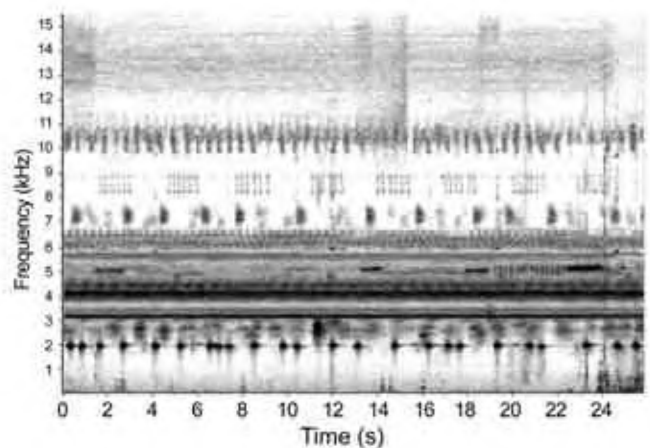
In contrast to *H. denticulata*, the signal of the field cricket *Eurygryllodes*, which was calling continuously at a distance of only 2 m from the preparation at a high sound intensity, did not obscure the representation of the temporal pattern of *M. marki*. The reason for this can be seen in the frequency tuning of the omega neuron, which is highly insensitive to sounds below about 7 kHz (Figure 8 d): since the calling song of *Eurygryllodes* has a frequency of 3.5 kHz, it is effectively filtered out by the nervous system of *M. marki* and does not interfere with the representation of the *M. marki* temporal pattern. Although the frequency tuning of *Eurygryllodes* was not examined, it is probable, from the general pattern in most field crickets, that its ear would be narrowly tuned around 3.5 kHz and it would be relatively insensitive to the higher frequencies of the other bushcricket species. The behavioural consequence is that *M. marki* and *Eurygryllodes* can call simultaneously within a short distance of each other and still avoid acoustic interference.

This principle of frequency partitioning between the calls of different species coupled with a matched frequency tuning in the corresponding receivers of each species has often been speculated to allow the co-existence of a large number of acoustically communicating species in an ecological community. That this is probably true can be seen in the spectrogram of ambient noise in a tropical evergreen forest at night (Figure 9): there are very well-defined and narrow frequency bands, many of them corresponding to the calls of single species (which may be recognized by unique temporal patterns).

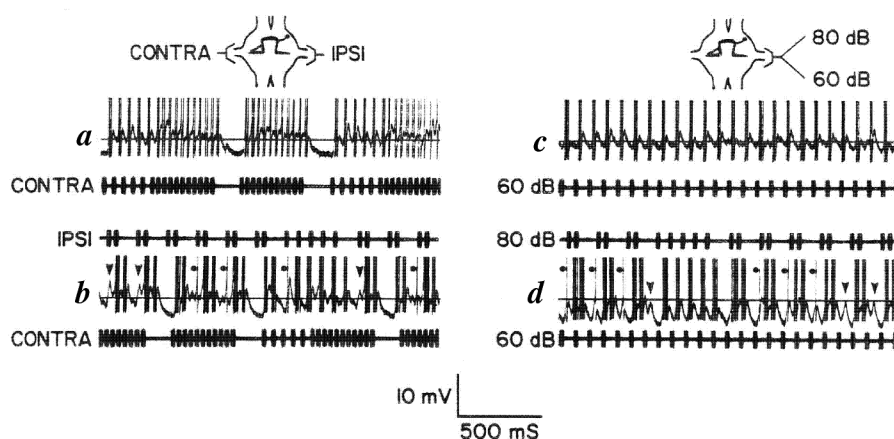
#### *Inter- and intra-specific interference: selective attention*

In addition to frequency tuning, auditory systems also use other mechanisms to deal with the problem of interference: one of the most interesting is selective attention. Using the tethered flight phonotaxis paradigm, Pollack

and Hoy<sup>8</sup> showed that crickets (female *T. oceanicus*) could reliably choose between two songs differing only in their temporal pattern presented simultaneously from the left and right side at equal intensities. This suggested that the crickets were able to represent the two song patterns independently in a side-specific manner, even though the song patterns themselves would be corrupted by interference at each ear (by the sound from the other side). Pollack<sup>38</sup> performed electrophysiological recordings in the laboratory from the omega neuron of *T. oceanicus*: he showed (Figure 10) that, when two temporal patterns were presented simultaneously from each side at equal intensities, the omega neuron clearly represented only the temporal pattern of the ipsilateral song (Figure 10 b; since there are a pair of omega neurons, one on each side, ipsilateral means the sound coming from the same side as the omega neuron being recorded). When the pattern on the contralateral side (see Figure 10) was played *alone* (Figure 10 a), the same omega neuron faithfully represented this temporal pattern, showing that, in the two-stimulus situation, the neuron was indeed somehow filtering out the contralateral pattern (Figure 10 a, b). Pollack<sup>38</sup> went on to show that this ‘selective attention’ to the ipsilateral stimulus was based on an intensity-dependent inhibitory input to the omega neuron, which effectively suppressed responses to the less intense of two stimuli, even if both were presented from the same side (Figure 10 c, d). By only representing the most intense stimulus in the omega neuron of each side, the mechanism of selective attention solves the problem of interference from signals in the same frequency range as that of the focal species. These interfering signals could stem from the calls of other species using the same frequency range or from simultaneously calling conspecifics.



**Figure 9.** Frequency partitioning in tropical forests. A spectrogram of nocturnal ambient noise recorded in a tropical evergreen forest ('Silent' Valley, Kerala, India) showing distinct and narrow frequency bands, some of which (at 2, 7 and 8–9 kHz) probably correspond to single species.



**Figure 10.** Neurophysiological basis of selective attention in crickets. The figure shows pairs of traces (in *a*, *b*, *c* and *d*) wherein the top (*a*, *c*) or middle trace (*b*, *d*) shows the activity of an auditory interneuron, the omega neuron, recorded intracellularly. For song patterns presented from one side (*a*, *c*), the oscillogram of the sound stimulus is shown below the neurophysiological recording; when presented from both sides simultaneously (*b*, *d*), oscillograms of the two sound stimuli are shown above and below the physiological recordings. Ipsi: stimulus presented from the same side as the omega neuron being recorded from; contra: stimulus from the opposite side as the omega neuron being recorded (see text for details). Note the preferential representation of the ipsilateral song pattern when two patterns are presented, one from either side (*b*) and of the louder pattern (*d*) when both are presented from the same side. Reprinted with permission from Ref. 38: Pollack 1988; Figure 1 (p. 2636); copyright 1988 by the Society for Neuroscience.

Males of a given cricket species often call within earshot of each other at relatively high intensities. In several species, they also form spatial aggregates or ‘choruses.’ A conspecific female walking through a chorus is thus confronted with several simultaneously calling males of her own species<sup>5</sup>. The calls of the different males can also interfere with each other to mask the species-specific temporal pattern so critical for song recognition<sup>35</sup>. Due to the different positions of the males, individual differences in song intensity, as well as stochastic influences such as wind, however, the different songs are unlikely to be at the same intensity on arrival at the ear. Selective attention can thus act to represent the most intense call: this was shown experimentally in the field for the bushcricket *Tettigonia*<sup>39</sup>. It follows then that any given male in a chorus usually hears only the most intense of several simultaneously singing neighbours on each side. Similarly, a female in a chorus of conspecific males represents in her nervous system, and therefore only perceives, the loudest male on each side. ‘Simultaneous sampling’ of several males, as sometimes postulated in models of mate sampling, is thus physiologically impossible: at any instant, the female cricket perceives, and therefore can sample, only two males. Sampling of further males can only be achieved sequentially by moving through the chorus<sup>39</sup>: this may be one reason why males, though singing within earshot of each other, do not usually space themselves too close to their nearest neighbours. Unless they are among the loudest singers in the population, there is a chance that they may not even be perceived by a female if their immediate neighbour happens to sing louder: where the female is concerned, only the loudest male on either side exists!

### Neurobiology and the ontogeny of acoustic communication

Acoustic communication in orthopteran insects appears to be mostly genetically pre-programmed: the circuits for song production can mature in cricket larvae in the absence of any auditory feedback<sup>40</sup> and there is so far little evidence for experience-dependent plasticity in the circuits mediating song recognition. That this is so is probably a consequence of the life history of most orthopteran insects: with their relatively short adult life span (of at most a few weeks), solitary lifestyle and lack of overlapping generations, there is little opportunity for song learning to evolve. In vertebrates, with their longer life spans and overlapping generations, the production of species-specific acoustic signals is not entirely pre-programmed and needs to be learned by each individual during its lifetime. The system that has been most thoroughly investigated in this regard has been the learning of song by birds and there exists a considerable literature on this that is beyond the scope of this review. I shall, however, draw a few examples from the study of song learning in birds to illustrate the connection between neurobiology and the ontogeny of behaviour.

Songbirds typically learn their songs in two phases: an early sensory phase when the bird requires to hear conspecific song, followed by a later phase of sensorimotor learning, when the bird actively sings and matches its vocal output to the stored template of song heard in the earlier phase<sup>41</sup>. The two phases may be separated in time (as in song sparrows) or may show overlap, as in zebra finches. A third phase may also be seen, whereby the final repertoire of the bird is crystallized<sup>41</sup>.

Songbirds possess a set of specialized brain nuclei that are apparently dedicated to the perception and production of song<sup>42</sup>. These nuclei are organized into two pathways (Figure 11): the first is a motor pathway extending from the HVC ('higher vocal centre'), which initiates premotor commands for singing via the RA (robust nucleus of the archistriatum) to the nXIIts that projects to the muscles of the syrinx (vocal organ). The HVC is also connected indirectly to the RA via an anterior forebrain (AF) pathway constituted by the Area X, DLM (medial part of the dorsolateral thalamus) and LMAN (lateral part of nucleus of

the anterior neostriatum). Most of the experiments on the neurobiology of song learning have been performed on zebra finches.

### *The anterior forebrain pathway and song learning*

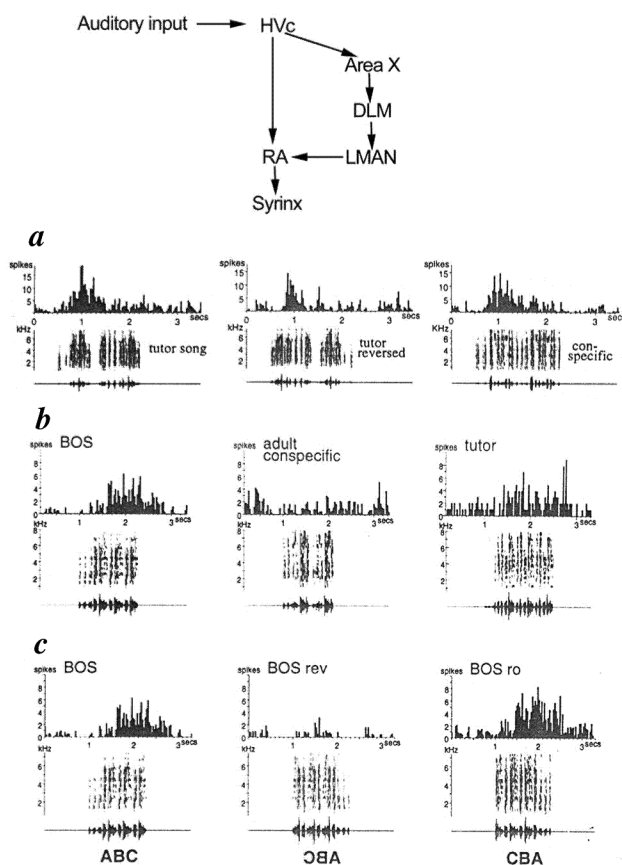
Whereas the motor pathway must obviously be intact for singing in adult zebra finches<sup>42</sup>, lesions of the AF pathway do not affect normal song production in adults. The AF nuclei must, however, be intact during the learning phase if a normal pattern of song production is to develop<sup>43</sup>. Interestingly, lesions of Area X prevent song crystallization, whereas lesions of LMAN cause premature crystallization of partially-learned songs, which are thus highly abnormal<sup>44</sup>. The premature crystallization caused by LMAN lesions may be due to its effects on the downstream nucleus, the RA: there is evidence that inputs from the LMAN are required for the maintenance of synaptic plasticity in the RA<sup>41</sup>.

On the other hand, specific pharmacological blocking of NMDA receptors (known to be involved in the formation of long term memory in mammalian systems) in the LMAN only during the initial sensory phase of exposure to tutor songs also results in very poor copying of the song at later stages, implicating the LMAN in the formation of the song template<sup>45</sup>.

Electrophysiological recordings from single neurons in several of the AF nuclei during playback of auditory stimuli have revealed the existence of neurons that are highly selective for complex temporal features of song<sup>46,47</sup>. Some respond selectively to the bird's own song and not to that of other conspecifics, some are sensitive to syllable order and yet others to particular combinations of syllables. Interestingly, this selectivity is absent in young birds, the AF neurons of which respond to a number of stimuli<sup>48</sup> (Figure 11). As the sensorimotor learning phase proceeds, single neurons are seen to develop their selectivity to the temporal features of the bird's own song<sup>48</sup>. Zebra finches thus provide neurobiologists with an excellent system to investigate the physiological process by which individual neurons develop selectivity for complex stimulus features in an experience-dependent manner.

### *Singing behaviour and neurogenesis*

The neural basis of sex-specific song production has been examined in the zebra finch, where only male birds sing. A comparative study of neural development revealed that there was no sexual dimorphism at birth, with both sexes possessing song nuclei at similar stages of development<sup>41</sup>. Within a few weeks of hatching, however, the song nuclei of males increased in size and cell number, whereas those of females regressed. Functional connections, particularly between the HVC and RA, were not completed in females<sup>49</sup>. Interestingly, the administration of oestrogen



**Figure 11.** The ontogeny of temporal pattern selectivity in single neurons of the auditory forebrain in zebra finches. Top: Schematic representation of the major brain nuclei involved in bird song production and perception (feedback loops omitted for simplicity). Each of the three graphs in *a*, *b* and *c* shows the activity (spiking response or number of action potentials) of a single neuron in the LMAN in response to stimulation with specific song models, the spectrograms and oscillograms of which are illustrated immediately below the neuronal responses. *a*, Non-selective response of an LMAN neuron from a 30-day-old juvenile zebra finch pre-exposed to a tutor song model: the neuron responds similarly to tutor song played forwards or backwards, and to the song of another conspecific. *b*, *c*, Recordings from two different LMAN neurons from 60-day-old zebra finches. The appearance of temporal selectivity is obvious. BOS: bird's own song; rev: reversed song; ro: reverse order of syllables. Adapted with author's permission from Ref. 48. Doupe and Solis 1997: Song- and order-selective neurons develop in the songbird anterior forebrain during vocal learning, Figs 4, 6. *b*, *c*, *d* reprinted with permission of Wiley Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Copyright © 1997 John Wiley & Sons, Inc.

during the early phase after hatching successfully prevented the atrophy of song nuclei in females, leading to the development of a functional song system<sup>49</sup> (oestrogen apparently causes masculinization of the brain when administered during early development). These females could in fact be induced to sing when adult upon administration of testosterone<sup>41</sup>.

The observation in both zebra finches and canaries that periods of active sensorimotor learning were correlated with increases in neuron number in song nuclei such as the HVC and Area X led to the postulate that learning involved or triggered an increase in neuronal numbers<sup>41</sup>. Canaries are open-ended learners and go through a plastic phase each spring where they incorporate new syllables and crystallize these novel songs<sup>42</sup>. The strong seasonality of the plastic phase is temporally correlated with seasonal increases in the volume of the HVC caused both by an increase in neuron size and number<sup>50</sup>. Does learning trigger the increase in cell size and number in an activity-dependent manner or does the increase in cell size and number enable the plastic phase of learning? The correlative nature of the above observations did not allow a distinction between cause and effect.

Using a comparative approach, Brenowitz *et al.*<sup>51</sup> showed that seasonal changes in HVC volume and cell size occurred in birds that were not seasonal learners, suggesting that the cell growth was not a consequence of learning but occurred independently of it, perhaps in response to seasonal variations in hormonal levels. In another experiment, wrens that were only allowed to learn a limited repertoire of five songs showed no difference in terms of increase in cell number in the song nuclei from those who were allowed to learn an extended repertoire of 45 songs<sup>52</sup>. This experiment also suggested that the increase in number of neurons was not an activity-dependent consequence of learning. On the other hand, there was a correlation between number of HVC neurons and the ability to learn new syllables in zebra finches, suggesting that the increase in neuron size and number may in fact enable song learning<sup>41</sup>.

Neural development and growth thus enables and constrains song learning; on the other hand, auditory feedback during sensorimotor learning serves to shape the physiological properties of neurons, allowing both the production and recognition of species-specific calls and individual repertoires.

### Neurobiology and the evolution of acoustic communication

Communication between individuals involves the production of signals by senders, which are perceived by receivers, who may then respond to these signals with specific behaviours. The reception and perception of signals, as well as the behavioural responses to these signals, are all

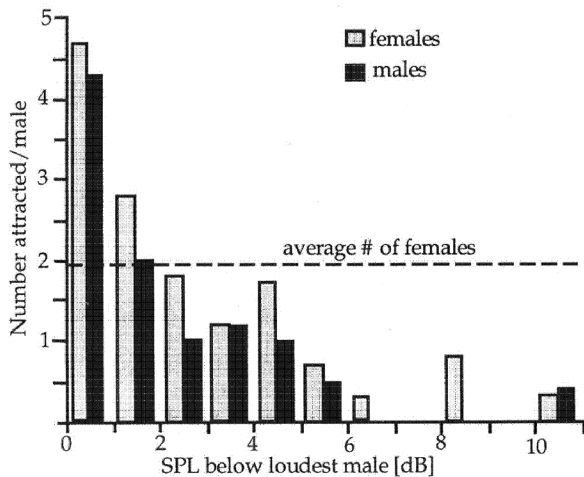
carried out by the nervous system. In acoustic communication, signal production is also under direct neural control. Acoustic communication may thus be looked upon as the interactions between the nervous systems of individuals. An understanding of the structure and function of the underlying neural circuitry is therefore required to understand these behavioural interactions. In addition, the physiological properties of these circuits can often provide insights and possible causal explanations for the direction of evolution of signals as well as behavioural responses of receivers.

The first indications of this were provided by the discovery of 'supernormal stimuli' by early ethologists, who found that highly exaggerated versions of normal stimuli produced extremely strong responses in receivers<sup>1</sup>. For example, gulls were found to incubate model eggs several times the size of their own eggs, even if they could hardly manage to sit on them! Similarly, herring gull chicks were found to preferentially peck for food at a red-and-white striped model in preference to that of a normal herring gull head (which has a white head and yellow beak with a red spot)<sup>1</sup>. These observations of apparently abnormal and maladaptive behaviour revealed that intense stimulation of the nervous system with the appropriate or key stimuli could produce very strong responses that could even over-ride responses to the corresponding natural stimuli. In present-day terminology, such behaviours are said to be the result of 'sensory biases'.

### Sensory biases and the evolution of signals

In several acoustically communicating species of frogs and insects, females are known to preferentially approach and mate with males that typically call louder, longer and at higher rates than other conspecific callers<sup>53</sup>. Although this observation is based on a huge body of evidence on several species<sup>53</sup>, I illustrate it here with an outdoor arena experiment by Forrest and Green<sup>54</sup> on a species of the mole cricket *Scapteriscus* (Figure 12): males that called just 2 dB (decibels) lower than the loudest attracted less than the average number of females, and those that called 6 dB lower attracted no females at all. The preference for louder and longer calls is probably a direct consequence of their greater power (energy per unit time), which elicits a stronger neural response, in terms of rate of action potentials, causing the receiver to turn towards and approach the most intense of the perceived stimuli. In species that use selective attention, another factor could be the lack of representation of the less intense signal in the nervous system of the receiver so that it is not even perceived. Receivers could thus be described as possessing a 'sensory bias' (the term 'neural bias' may be preferable) for more intense stimuli, which would result in directional selection on senders to produce more intense signals at high rates<sup>53</sup>.





**Figure 12.** Female preference for intense signals. The graph shows the number of free-flying mole crickets (*Scapteriscus acletus*) attracted per calling conspecific male in an outdoor arena experiment. Males singing at sound pressure levels (SPL) of 2 decibels (dB) lower than the loudest attract less than the average number of females and those singing 6 dB lower attract almost no females. Interestingly, conspecific males are also attracted, which may result in the formation of male aggregates. Reprinted with permission from Ref. 54: Forrest and Green 1991, Figure 3 (p. 100); copyright 1991 AB Academic Publishers, Oxon.

In addition to the physiological properties of the receiver system, the direction of evolution of signals is also determined by their adaptive value. In the case of selection for intense signals, the loudest signals may be produced by the largest males<sup>54</sup>, who are also the most likely to provide the greatest fitness benefits to females. In some species of crickets, for example, mating with larger males results in the production of a greater number of offspring<sup>55,56</sup>. Since calling is also energetically expensive, signal intensity, duration and calling rate could serve as honest indicators of male quality. In the case of selection for higher intensity signals, therefore, the sensory bias could be correlated with positive fitness consequences.

Ecological factors also influence the direction of evolution of signals: these include the transmission properties of the habitat and the presence of acoustically orienting predators or parasitoids<sup>35</sup>. Since the habitat typically attenuates sounds, intense signals would be selected for in order to improve detectability and transmission distance<sup>35</sup>. On the other hand, acoustically orienting predators and parasitoids select against the evolution of intense signals and, in fact, there are demonstrated instances of selection against high rates and duration of calling, as well as a switch to other, less conspicuous modes of communication, such as vibrational signalling<sup>57</sup>.

The evolution of *complex* signals may also be explained in terms of sensory biases: this was exemplified in a classic study on acoustic communication and sexual selection in the tungara frog, *Physalaemus pustulosus*<sup>58</sup>. This frog species uses acoustic signals for mate attraction

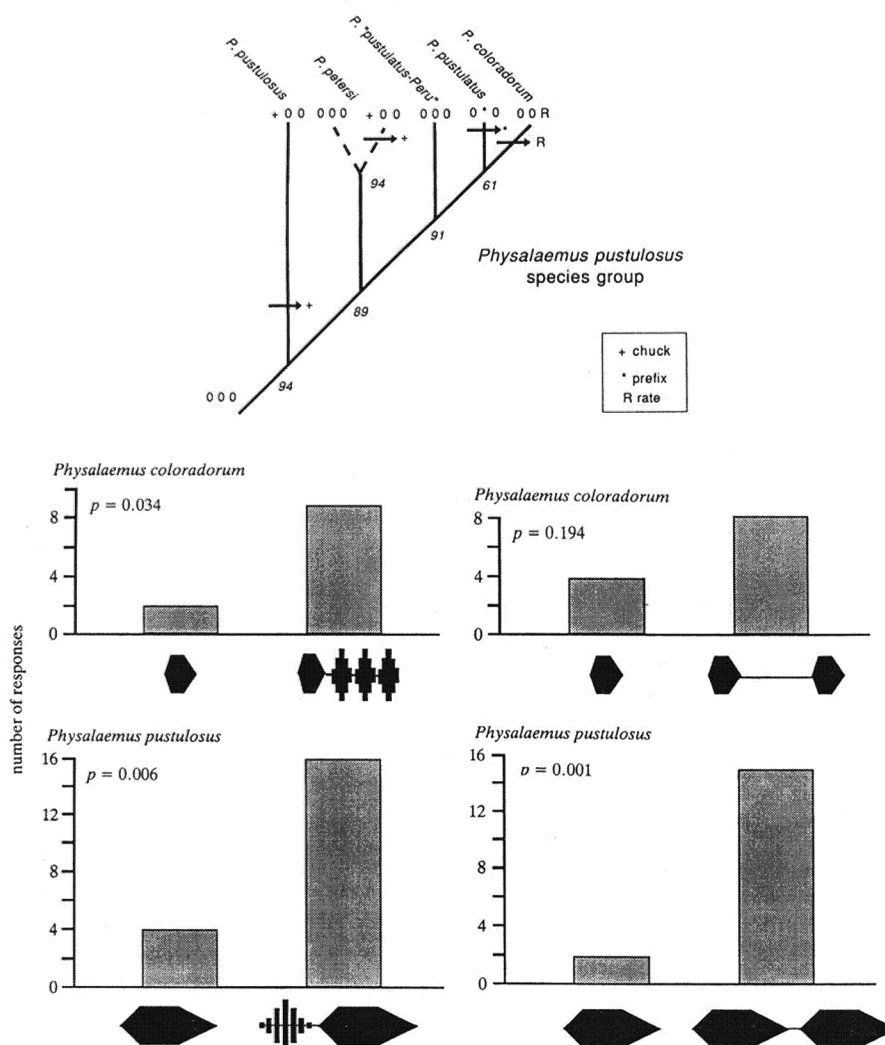
and has a two-part call, consisting of a whine followed by a higher frequency component called the chuck. Ryan<sup>58</sup> found that the whine was both necessary and sufficient to attract females but whines with chucks added were more attractive than the whine alone. Frogs did not always use the chuck component (which also attracted bat predators), but added them in the presence of rival males<sup>58</sup>.

Using a phylogenetic approach, Ryan and Rand<sup>59</sup> examined the evolution of both male call structure and female preference in the *P. pustulosus* species group (Figure 13). They found that all five species in the group produced whine-like calls, whereas a chuck-like suffix had evolved independently of *P. pustulosus* in only one other species, *P. petersi*. Another species, *P. pustulatus*, had an amplitude-modulated prefix to the whine (Figure 13). Using playback experiments, they demonstrated that the preference for chucks existed in females of species in which male calls did not contain chucks (Figure 13). Also, *P. pustulosus* females responded strongly to calls consisting of the conspecific whine together with either the AM prefix of the *P. pustulatus* call or in fact of a number of different kinds of suffixes, including white noise<sup>60</sup>!

Taken together, these experiments demonstrated that the preference for chucks predated the evolution of the chucks themselves and had not evolved in a process of sender–receiver co-evolution<sup>59</sup>. The preferences for the chucks constituted sensory biases that could have driven the evolution of these additional components. Secondly, the preferences were not confined to chuck-like patterns and several other patterns could substitute for the chuck. The exact structure of the various call prefixes and suffixes that had evolved within the group was probably a matter of chance<sup>59,60</sup>. Call complexity appears to have evolved several times independently within the group in response to this latent bias in the nervous system. The bias for complex calls could have arisen because these calls cause greater stimulation of the auditory system than the simple ones at physiologically achievable sound intensities, i.e. in order for a simple call to cause the same amount of stimulation as a complex call, it would need to be louder by about 150%, which is probably physiologically impossible<sup>61</sup>.

### Sensory biases and the evolution of behaviour

One of the most striking aspects of anuran and insect communication behaviour is the phenomenon of synchronous signalling, wherein a number of individuals produce their signals at exactly the same time. Compelling examples include the synchronous flashing of fireflies and the acoustic choruses of cicadas and bushcrickets. Why do individuals synchronize their signals? Several hypotheses have been put forward<sup>62</sup>: synchrony has been proposed to be a strategy to minimize inter-individual acoustic interference and thus preserve the species-specific temporal



**Figure 13.** Sensory biases for novel acoustic stimuli in frog species of the genus *Physalaemus*. The upper figure shows the phylogeny of the *Physalaemus pustulosus* species group and the pattern of call evolution. R rate = call repetition rate. Numbers in italics represent bootstrap confidence intervals. The graphs below illustrate the phonotactic responses of females (of the species indicated in each case) in two-stimulus choice experiments. The addition of novel suffixes and prefixes to the species-specific call renders it more attractive in most cases (see text for details). Reprinted with permission from Ref. 59: Ryan and Rand 1993 'Sexual selection and signal evolution: the ghost of biases past' Figs 2, 4 (p. 190, 192); copyright 1993 The Royal Society.

pattern. Where the spatial distribution of calling males is a pattern of clumped aggregates, it has been argued that groups that synchronize their signals and increase their signal intensity are more likely to attract females. None of these hypotheses are supported by any empirical evidence.

The calling songs of the tettigoniid species *Neoconocephalus spiza* and *Mecopoda elongata* both consist of discontinuous chirps produced at rates of about 3.5 per second in *N. spiza*<sup>63</sup> and one every two seconds in *M. elongata* (Roemer, personal communication). Males of both species synchronize their chirps on a call-by-call basis with simultaneously-calling conspecifics<sup>63,64</sup>. Since the neural circuits that are responsible for song production in crickets appear to behave as oscillators<sup>40</sup>, the call-

ing interactions may be described in terms of coupled oscillators. Greenfield<sup>63</sup> proposed a model of inhibitory resetting of oscillators as a mechanism by which two interacting males of *N. spiza* could achieve synchrony on a chirp-by-chirp basis. Even if inhibitory resetting could provide a mechanistic explanation of how synchrony was achieved, the question of how such a mechanism could have evolved was still left open.

The following experiment provided the clue to a possible explanation: when *N. spiza* females were given a choice between two simultaneously presented chirps (one from each side), of which one was of longer duration than the other, the female, not unexpectedly, preferred the longer chirp<sup>65</sup>. But when the relative timing of the two chirps was adjusted so that the shorter chirp preceded the

longer one from 50 to 10 ms, the preference of the female shifted abruptly to the shorter one. This tendency to preferentially orient towards and approach the leading of two sounds presented in quick succession is termed the precedence effect. Psychoacoustic experiments in humans have also demonstrated that we tend to perceive only the leading of two sound stimuli presented in quick succession from opposite sides<sup>66</sup>.

The neural basis of the precedence effect has been investigated in detail in the other synchronizing bushcricket species, *Mecopoda elongata*, which also exhibits the precedence effect<sup>64</sup>. By simultaneously recording the electrophysiological activity of a pair of auditory interneurons (the omega neurons) that are known to inhibit each other, Roemer *et al.*<sup>64</sup> showed that the leading of two chirps presented at time delays between 5 and 120 ms was clearly represented in the omega neuron on that side, whereas the representation of the lagging chirp in the contralateral omega neuron was strongly suppressed. Reciprocal contralateral inhibition is a very common mechanism of contrast enhancement in nervous systems, typically used in auditory systems for sound localization. Thus, the neural mechanism of sound localization acts as a sensory bias that is responsible for the female preference for leading signals.

Due to the precedence effect, it follows that, for males calling at similar intensities, those who could produce calls that were slightly in advance of but overlapping with those of their nearest neighbours, would be likely to attract more females. Males that paid attention to their neighbours and reset their calling rates to lead their neighbours would thus be selected for, leading to the evolution of inhibitory resetting mechanisms. Using this logic, Greenfield *et al.*<sup>67</sup> developed a simulation wherein they demonstrated that synchrony could evolve as an epiphenomenon of intermale competition to produce leading calls. The selection pressure to produce leading calls was due to the female preference for leading calls, itself a consequence of the strategy of reciprocal contralateral inhibition for contrast enhancement to allow better sound localization. The evolution of chorusing thus provides an illustrative example of how inherent biases within the nervous system, which exist as a result of computational requirements, could drive the evolution of novel patterns of behaviour.

### Neurobiology and behaviour: a network of connections

The several examples above illustrate the intricate connections between neurobiology and behaviour: each is indispensable to the understanding of the other. Studies of individual behaviour that incorporate both behavioural and neurophysiological approaches often result in more reliable and robust conclusions than those that utilize either

alone. This is exemplified by the case of courtship communication in crickets. Communication during courtship is multimodal, with both acoustic and contact chemosensory cues being used by females to make mating decisions<sup>68,69</sup>. Additional cues provided by male courtship displays include vibrational signals and the air puffs produced by the forewings during singing<sup>70</sup>. These wind puffs are sensed by receptors situated on the cerci. Electrophysiological experiments revealed that the temporal pattern of cricket courtship song was represented in the activity patterns of abdominal interneurons that were downstream of cercal receptors<sup>70</sup>. For several years, this was taken as evidence of a communicatory role for air-borne wind puffs during courtship. Behavioural experiments designed to explicitly test this showed that these air-borne windpuffs produced during courtship stridulation are unlikely to play a role in courtship communication<sup>71</sup>.

On the other hand, the rules of neural processing and organization inferred from behavioural observations and experiments must be verified electrophysiologically. Behavioural paradigms are extremely useful in order to narrow down the relevant signal components of the stimulus (key or sign stimuli), the rules of processing of these components and their anatomical localization. They allow neurobiologists to home in on circuits with interesting and relevant physiological and computational properties. An understanding of these properties in turn allows a better understanding of the behaviour in question in a feedback manner.

An understanding of the neural basis of behaviour also provides important insights into its ecological and evolutionary aspects, as amply illustrated in this article. On the other hand, physiological processing mechanisms often become meaningful only when placed in the context of the ecology and evolution of the organism. Physiology, ontogeny, evolutionary history and adaptive value: the four faces of behaviour envisaged decades ago by Niko Tinbergen. A neurobiological viewpoint surely increases the clarity of our perception of each of these aspects.

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